Comparison of the Beckman Auto ICS and the Syva Autolab 6000 for Determination of Gentamicin Levels in Serum

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Two fully automated drug-monitoring systems, the Syva Autolab 6000 and the Beckman Auto ICS, were compared in terms of accuracy, precision, speed of operation, and cost effectiveness in the determination of gentamicin levels in serum. Within-run and between-run precision for both systems were acceptable (coefficient of variation, 2.0 to 6.9%), and patient sample comparisons resulted in an intermethod correlation coefficient of 0.96. When reference samples (prepared to contain 1.2 to 10 μg of gentamicin per ml) were assayed, the Syva Autolab 6000 obtained concentrations within 8% of expected values, whereas the Beckman Auto ICS reported values up to 17% higher than target values. In a time and cost comparison, reagent costs for the Beckman system were ca. 50% less than for the Syva Autolab 6000; the Syva system, however, determined patient results two to three times faster than the Beckman Auto ICS.

Gentamicin is a potent aminoglycoside antibiotic often used in the treatment of severe gram-negative bacillary infections. For safe and efficacious therapy to be achieved, gentamicin levels in serum must be maintained within a narrow concentration range so as to allow the infecting organism to be killed while minimizing nephrotoxic or ototoxic side effects to the patient (1, 5, 9). Due to varying aminoglycoside elimination rates among patients, prediction of adequate gentamicin levels in serum based solely on calculated dosages is often misleading, reinforcing the need for laboratory monitoring of gentamicin levels in serum (2, 10, 13).

Many methods are currently available for measuring aminoglycoside levels, including bioassay, radioimmunoassay (RIA), high-pressure liquid chromatography, enzyme immunoassay, and fluorescent immunoassay (3, 4, 8, 11, 12). For clinical laboratories with large drug-monitoring work loads, fully automated systems have been developed which significantly reduce technologist time and generate results faster. The Autolab 6000, an automated system recently introduced by Syva Co., uses a homogeneous enzyme-linked immunoassay technique (EMIT) which has been shown to be accurate, specific, and easy to perform (6–8, 11). Beckman Instruments, Inc., has upgraded its ICS Analyzer II to an Auto ICS, an automated system which uses a rate nephelometric inhibition immunoassay to perform therapeutic drug determinations.

The purpose of the present study was to compare two fully automated systems (Syva Autolab 6000 and Beckman Auto ICS) in terms of accuracy, precision, speed of operation, and cost effectiveness in the measurement of gentamicin levels in serum. (This paper was presented in part at the 51st Annual Meeting of the American Society for Medical Technology, Los Angeles, Calif., 12 to 17 June 1983.)

MATERIALS AND METHODS

Patient samples. Serum samples were collected routinely from patients at Worcester Memorial Hospital, Worcester, Mass., who were receiving therapy with gentamicin. All samples were assayed initially within 8 h after collection by the Syva EMIT method and then frozen at −70°C in stoppered glass tubes. Of these frozen samples, 41 sera, representing a wide range of concentrations from collection over a 6-week period, were thawed and assayed within 4 h by the Beckman Auto ICS and simultaneously reassayed by the Syva Autolab 6000. All patient samples were assayed in duplicate.

Reference samples. Gentamicin sulfate powder (potency, 568 μg/mg; Schering Corp., Kenilworth, N.J.) was dissolved in pooled human sera and then diluted to final stock concentrations of 1.2, 2.5, 5.0, 10.0, and 20.0 μg/ml. Portions of each stock solution were stored for 2 weeks in screw-capped glass vials at −70°C until simultaneous assay in triplicate by the Syva Autolab 6000 and the Beckman Auto ICS, as well as by an RIA technique included as a reference method. The RIA was performed in an outside laboratory with Gamma-coat (125I) gentamicin RIA kits (Clinical Assays, Div. of Travenol Laboratories, Inc., Cambridge, Mass.) according to the directions of the manufacturer.

Autolab 6000. Gentamicin assay kits for EMIT were purchased from Syva Co., Palo Alto, Calif. In EMIT, an unknown quantity of sample drug competes with a known amount of an enzyme-labeled drug for specific antibody binding sites. As enzyme-labeled drug binds to antibody, the activity of the enzyme is reduced, such that there is a direct correlation between the amount of sample drug being measured and the residual enzymatic activity. The amount of active enzyme remaining determines the rate of conversion of NAD to its reduced form (NADH), resulting in an absorbance change which is measured spectrophotometrically.

The components of the Autolab 6000 used to perform EMIT include an EMIT Autocarousel (Syva Co.) which automatically pipettes, mixes, and aspirates samples into the spectrophotometer from a 40-position turntable; a Gilford Stasar III spectrophotometer (Gilford Instrument Laboratories, Inc., Oberlin, Ohio) which measures the absorbance of the reaction mixture at 340 nm in a 30°C thermally regulated flow cell; and a Syva Lab Processor 6000 (manufactured for Syva Co. by Hewlett Packard Co., Corvallis, Oreg.) which plots and stores standard curves and calculates results.

* Corresponding author.
TABLE 1. Results of between-run and within-run precision measurements

<table>
<thead>
<tr>
<th>Assay method</th>
<th>Conc of gentamicin (µg/ml)</th>
<th>n</th>
<th>Mean (±SD) (µg/ml)</th>
<th>Range (µg/ml)</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beckman Auto ICS</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between run</td>
<td>2.3</td>
<td>15</td>
<td>2.7 (±0.18)</td>
<td>2.3-3.2</td>
<td>6.9</td>
</tr>
<tr>
<td>Between run</td>
<td>6.0</td>
<td>15</td>
<td>6.3 (±0.37)</td>
<td>5.3-7.1</td>
<td>5.8</td>
</tr>
<tr>
<td>Within run</td>
<td>2.3</td>
<td>10</td>
<td>2.6 (±0.05)</td>
<td>2.5-2.6</td>
<td>2.0</td>
</tr>
<tr>
<td>Within run</td>
<td>6.0</td>
<td>20</td>
<td>5.8 (±0.17)</td>
<td>5.3-6.0</td>
<td>2.9</td>
</tr>
<tr>
<td>Syva Autolab 6000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Between run</td>
<td>2.3</td>
<td>15</td>
<td>2.2 (±0.08)</td>
<td>2.0-2.3</td>
<td>3.8</td>
</tr>
<tr>
<td>Between run</td>
<td>6.0</td>
<td>15</td>
<td>5.8 (±0.16)</td>
<td>5.5-6.0</td>
<td>2.8</td>
</tr>
<tr>
<td>Within run</td>
<td>2.3</td>
<td>10</td>
<td>2.2 (±0.07)</td>
<td>2.2-2.4</td>
<td>3.2</td>
</tr>
<tr>
<td>Within run</td>
<td>6.0</td>
<td>10</td>
<td>5.8 (±0.14)</td>
<td>5.6-6.0</td>
<td>2.5</td>
</tr>
</tbody>
</table>

Standard curves were determined once each working day by assaying the 1.0- and 16-µg/ml calibrator samples in duplicate according to the Syva recommended protocol.

**Auto ICS.** A rate nephelometric inhibition immunoassay is the basis for therapeutic drug monitoring on the Beckman Auto ICS (Beckman Instruments, Inc., Brea, Calif). Free drug in the test sample competes with a known amount of drug-protein conjugate for a fixed number of antibody binding sites. Since only the drug-protein conjugates which bind to antibody can form light-scattering complexes that are detected by a rate nephelometer, there is an inverse relationship between the concentration of sample drug being assayed and the intensity of scattered light.

Beckman Instruments has recently upgraded its manual ICS Analyzer II to an Auto ICS, providing a fully automated system. The Auto ICS consists of the Immunochemistry Analyzer II, a rate nephelometer which measures the intensity of light scattered during the immunoprecipitation reaction; an automator module which automatically pipettes, dilutes, and dispenses samples into the reaction flow cell from a 40-position turntable; and a data processor which stores the calibration curve and calculates results. Immediately before each run of patient samples, the system was calibrated by assaying a single-level calibrator in triplicate (two of three measurements had to duplicate within ±5%) according to the Beckman recommended protocol.

Initially, repeated problems were experienced by our laboratory with the Auto ICS: difficulty in calibration of the Auto ICS, less than satisfactory reproducibility of results, and significantly higher concentration values obtained with the Auto ICS than with the Syva Autolab 6000. Upon assay of 76 patient samples by both the Beckman Auto ICS and the Syva Autolab 6000, we found an intermethod correlation coefficient of 0.93. When reference samples prepared to contain 1.2 to 10.0 µg of gentamicin per ml were assayed on the Beckman Auto ICS, recovered values were up to 32% higher than target values. To correct these difficulties, Beckman Instruments made several adjustments in the system. First, the pipettor module track, responsible for transporting the cars that dispense reagents and transfer samples to the flow cell, was realigned. Second, a valve was added which controls the rinsing of the flow cell with diluent between samples to rid the cell of variable amounts of polyethylene glycol, contained in the buffer, which can accumulate and alter the antigen-antibody reaction rate. The results presented in this report indicate the performance of the Auto ICS after modification of the system.

**Statistical analysis.** Correlation between methods (r) was evaluated by linear regression by the least-squares method. The coefficient of variation (CV) was calculated by dividing the standard deviation by the mean and then multiplying by 100.

**RESULTS**

**Precision.** Between-run precision was evaluated for the Beckman Auto ICS and for the Syva Autolab 6000 by assaying control samples (ICT: Scientific, Fountain Valley, Calif.) containing 2.3 and 6.0 µg of gentamicin per ml on 15 separate days over a 3-month period (Table 1). Both controls also were assayed 10 to 20 times within the same run to determine within-batch reproducibility (Table 1). The CVs for the Syva system (CV, 2.5 to 3.8%) generally were lower than for the Beckman system (CV, 2.0 to 6.9).

**Accuracy.** The triplicate readings obtained from simultaneous assay of the spiked gentamicin samples were averaged and then used to determine percent recovery values for the Syva Autolab 6000, the Beckman Auto ICS, and the RIA reference method (Table 2). Gentamicin recovery results were within 8% of the target values for the Syva Autolab 6000 and within 9% of expected values for the RIA method over the entire concentration range studied. With the Beck-

TABLE 2. Results of gentamicin recovery measurements

<table>
<thead>
<tr>
<th>Spiked conc (µg/ml)</th>
<th>Recovered conc (µg/ml)</th>
<th>% Recovery</th>
<th>Recovered conc (µg/ml)</th>
<th>% Recovery</th>
<th>Recovered conc (µg/ml)</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Beckman Auto ICS</td>
<td></td>
<td>Syva Autolab 6000</td>
<td></td>
<td>RIA</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>24.2</td>
<td>121</td>
<td>&gt;17.6</td>
<td></td>
<td>&gt;16</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>11.4</td>
<td>114</td>
<td>9.8</td>
<td>98</td>
<td>10.9</td>
<td>109</td>
</tr>
<tr>
<td>5.0</td>
<td>5.3</td>
<td>106</td>
<td>5.0</td>
<td>100</td>
<td>5.0</td>
<td>100</td>
</tr>
<tr>
<td>2.5</td>
<td>2.7</td>
<td>108</td>
<td>2.6</td>
<td>104</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>1.2</td>
<td>1.4</td>
<td>117</td>
<td>1.3</td>
<td>108</td>
<td>1.2</td>
<td>100</td>
</tr>
</tbody>
</table>
Determination of patient results included measurement of gentamicin concentrations in two controls and in patient samples run in duplicate and calculating final results. In the cost analysis (Table 4), cost of consumables was not tabulated because these expenses were equal and minor for both systems.

The Syva Autolab 6000 reports patient results two to three times faster than the Beckman Auto ICS. With the current price structure, reagents are ca. 50% less for the Beckman Auto ICS, with costs of $52.00 and $28.75 for the Syva and Beckman systems, respectively, when 10 patient samples were assayed as a batch.

**DISCUSSION**

If a CV of $\leq 10\%$ is considered acceptable performance in the comparison of assay methods for the clinical laboratory (8), the demonstrated between-run precision for the Syva Autolab 6000 (CV, 2.8 to 3.8%) and Beckman Auto ICS (CV, 5.8 to 6.9%) was acceptable for clinical application and was in general agreement with the findings of other investigators. Between-run precision studies with the manual Syva EMIT systems to measure gentamicin concentrations, O'Leary and co-workers (6) obtained CVs of 2.7 to 3.9%, whereas Ratcliff and co-workers (8) found CVs of 2.6 to 4.2%. Witebsky and associates (12) obtained between-run CVs of 3.7 to 13.4 and 8.3 to 16.8% for their Syva system and Beckman Auto ICS, respectively, when evaluating the reproducibility of four gentamicin assays. These investigators experienced precision problems similar to ours with the Beckman Auto ICS and noted an improvement in precision after the manufacturer modified the instrument.

For within-run precision, Witebsky and co-workers (12) obtained a CV of 8.6% during the performance of tobramycin studies on the Beckman Auto ICS before adjustments were made to the system and a CV of 3.8% with tobramycin level determinations after modification of the Beckman Auto ICS. Similarly, we found a notable difference in within-run CVs between the Beckman Auto ICS initially (CV, 6.3 to 6.6%) and after technical adjustments had been made (CV, 2.0 to 2.9%). Within-run precision for the Syva Autolab 6000 was excellent as indicated by CVs of 2.5 to 3.2% and was in agreement with the findings of O'Leary et al. (6), who obtained CVs of 1.7 to 3.1% with a manual EMIT procedure to measure gentamicin concentrations.

In terms of accuracy, Delaney and co-workers (4) found 86 to 92% recovery when assaying spiked gentamicin concentrations ranging from 2.2 to 32.7 $\mu$g/ml with a semiautomated Syva system. In our current study, the Syva Auto-
lab 6000 demonstrated gentamicin recovery values within 8% of target values over the concentration range of 1.2 to 10.0 μg/ml. In contrast, the gentamicin recovery results obtained by us with the Beckman Auto ICS were up to 17% higher than the expected values. Comparison of patient serum samples assayed under the same conditions by both the Syva Autolab 6000 and the Beckman Auto ICS indicated a good correlation between the two systems (r, 0.96; slope, 1.01).

Our time analysis indicates that technologist hands-on time for both systems is comparable. A notable difference between the two systems is found in the time needed to analyze a sample and report a final result, with the Beckman system requiring two to three times longer than the Syva system. However, with present price structure, reagent costs for the Beckman Auto ICS are ca. 50% less than for the Syva Autolab 6000. If the expenses for both reagents and time required to obtain final results are considered together, operation of the slower Beckman Auto ICS is ca. 20% less costly than the Syva Autolab 6000.

ACKNOWLEDGMENTS

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LITERATURE CITED